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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF PREVENTION, PESTICIDES, AND TOXIC SUBSTANCES
WASHINGTON, D.C. 20460

DATE: February 23, 2000

MEMORANDUM

SUBJECT: ***DINOCAP*** - Report of the Hazard Identification Assessment Review Committee.

FROM: Paul Chin
Reregistration Branch I
Health Effects Division (7509C)

THROUGH: Jess Rowland, Co-Chairman
Hazard Identification Assessment Review Committee
Health Effects Division (7509C)
and
Pauline Wagner, Co-Chairman
Hazard Identification Assessment Review Committee
Health Effects Division (7509C)

TO: Catherine Joseph, Risk Assessor
Reregistration Branch I
Health Effects Division (7509C)

PC Code: 036001

On December 1, 1999, the Health Effects Division's Hazard Identification Assessment Review Committee (HIARC) evaluated the toxicology database of **dinocap** and selected the toxicological endpoints for acute and chronic dietary, occupational, and residential (dermal and inhalation) exposure risk assessments. During this meeting, one issue, which required further evaluation of the available data, was unresolved. The issue was to confirm whether an increase in the duration of gestation in treated CD–1 mice at all dose levels in a developmental toxicity in mice was toxicologically significant. David Anderson was requested to verify the results of this developmental toxicity. After reviewing the historical control data, the re-evaluation of the statistical analyses and the manner in which the gestational length (days) were recorded, the increase in gestational time was not considered to be toxicological significance in the mice developmental toxicity in study (MRID# 41313001; HED Document# 008299). This reviewer agrees with the conclusions derived by the study reviewer, Joint Meeting on pesticide Residues (JMPR), and the registrant that the NOAEL for developmental toxicity is 4 mg/kg/day; the LOAEL is 10 mg/kg/day based on the slight (non-significant) increase in incidences of cleft

palate and eyelids-open relative to the controls. The NOAEL for maternal toxicity is 10 mg/kg/day; the LOAEL is 25 mg/kg/day based on the slight decrease in body weight and body weight gains. The Committee's conclusions are presented in this report.

Committee Members in Attendance

December 1, 1999

Members present were David Anderson, Bill Burnam, Virginia Dobozy, Pamela Hurley, Mike Ioannou, Tina Levine, Sue Makris, Nancy McCarroll, Nicole Paquette, Kathleen Raffaele, Jess Rowland, PV Shah, Pauline Wagner, and Brenda Tarplee (Executive Secretary). Members in an absentia was Karen Hamernik. Data were presented by Paul Chin of Reregistration Branch I. Also, in attendance were Whang Phang, Catherine Joseph, Elizabeth Mendez, Vicki Dellarco, and Karl Baetcke.

Data Presentation: and Report Presentation	_____ Paul Chin Toxicologist

I. INTRODUCTION

There are no current food uses in the U. S. It is registered only for greenhouse ornamentals. The registrant wants to maintain the tolerances on grapes and apples. Since the registrant wants to maintain the import tolerances, dinocap will be treated as a food use chemical.

Chemically, technical dinocap is a mixture of dinitro-octylphenylcrotonate isomers and dinitro-octylphenols. Toxicological database shows that there are two purities (76-88% a.i. and 92-96% a.i.) for the technical dinocap. The older studies were conducted mainly with 76-88% a.i. and the newer studies were conducted with 92-96% a.i.. The amount of dinitro-octylphenols in 76-88% and 92-96% dinocap constitutes 6% and 0.5% of the a.i., respectively. **2,4-Dinitrophenol** is well known developmental toxicant. There is evidence to suggest that technical dinocap is a developmental toxicant in rabbits and mice.

The HED RfD/ Peer Review Committee (HED Doc. No. 011076 dated June 24, 1994) considered that the carcinogenicity phase of the rat study (MRID No. 41065401; ACCESSION No. 00247959) and the carcinogenicity study in mice (MRID Nos. 418639801, 42079102) were considered to be adequate. The high dose levels tested in both rats and mice carcinogenicity studies were considered to be adequate for carcinogenicity testing. The dinocap treatment did not alter the spontaneous tumor profile in either animal species. The chemical was classified as a **"Group E"**.

II. HAZARD IDENTIFICATION

A. Acute Reference Dose (RfD) Subpopulation (Females 13+)

Study Selected: Developmental Toxicity Study in Mice **Guideline #:** 83-3(a)

MRID No.: 41313001

Executive Summary:

In a developmental toxicity study (MRID No. 41313001), Dinocap (94.4% a.i.) suspension in an aqueous 1% Tragacanth Gum was administered by gavage to CD-1 (ICR) BR mice (24/dose) at 0, 4, 10 or 25 mg/kg/day from gestation days 6 through 15. One group of 12 presumed pregnant females underwent caesarean section while the other 12 pregnant females were allowed to deliver naturally.

There were no maternal deaths. No clinical signs or post mortem observations were attributed to test article administration. Although not statistically significant, the body weight in the high-dose animals (25 mg/kg/day) was 5% lower than the controls on day 18 of gestation. In this group, the body weight gains were 7 and 11% lower than the controls from gestation days 6-15 and 0-18, respectively. **The NOAEL for maternal toxicity is 10 mg/kg/day; the LOAEL is 25 mg/kg/day based on the slight decrease in**

body weight and body weight gains.

Although not statistically significant, dinocap at 25 mg/kg/day caused the following: reduction in numbers of corpora lutea, implantation size, litter size, and the percent of dams with any resoprtions. The number of total resorption and dead or resorbed conceptuses/litter in the high-dose animals were increased ($p<0.05$) when compared to the controls. In addition, dinocap at 25 mg/kg/day caused the following when compared to the controls: reduction in live fetal body weights (29% less than the controls, $p<0.01$); increased incidence of cleft palate ($p<0.01$), "eye lids open" ($p<0.01$), and head tilt; and an effect on swimming performance ($p<0.01$) (as measured by mice which sank and required rescue or swam on their side).

In the 10 mg/kg/day group, the number of total resorption was increased ($p<0.05$) and live fetal body weights were decreased (7% lower than the controls, $p<0.05$). However, these differences from control groups are considered to be of little or no toxicological significance. In the 10 mg/kg/day group, in addition, a slight (non-significant) increased incidences in cleft palate and eyelids-open occurred relative to the control group. Due to the absence of any occurrence in either concurrent or historical controls, the NOAEL is set conservatively at 4 mg/kg/day.

Natural delivery data showed that viability index, number of live precull on day 4 divided by number of live on day 1, was decreased ($p<0.01$) at 25 mg/kg/day (87% versus 97-100%) in the control and two lower dose groups. Lactation index was reduced ($p<0.01$) in the 4 mg/kg/day group only. However, this was primarily due to 8 pups from one litter dying by day 7 of weaning.

The NOAEL for developmental toxicity is 4 mg/kg/day; the LOAEL is 10 mg/kg/day based on the slight (non-significant) increase in incidences of cleft palate and eyelids-open relative to the controls.

This study is classified as ACCEPTABLE/NONGUIDELINE and does not satisfy the guideline data requirement for a developmental study (83-3a) in mice. The study was designed to answer specific question (the inner ear formation). The Agency requested that the registrant perform a "modified" developmental toxicity study in mice in order to elucidate developmental toxicity potential of purified dinocap (Q. Bui of the Agency to D. Edwards, dated 10/19/88, Tox. Doc. No. 007457).

Dose and Endpoint for Risk Assessment: The NOAEL for developmental toxicity is 4 mg/kg/day; the LOAEL is 10 mg/kg/day based on the slight (non-significant) increase in incidences of cleft palate and eyelids-open relative to the controls.

Uncertainty Factor(s): An uncertainty factor of 100 was applied to account for inter-species extrapolation (10 x) and intra-species variability (10 x).

ACUTE RfD (females 13+) :

$$\frac{4 \text{ mg/kg (NOAEL)}}{100 \text{ (UF)}} = 0.04 \text{ mg/kg}$$

Comments about Study/Endpoint/Uncertainty Factor(s): This endpoint is appropriate for females 13+ subpopulation only since the end point is an in utero affect.

There is evidence to suggest that **purified** dinocap (94.4% a.i.) is a developmental toxicant in mice at doses which were not maternally toxic (see executive summary described above). However, **technical** dinocap (84% a.i.) is also a developmental toxicant in mice at doses which were maternally toxic (Rogers J. M. et al. 1986, EPA's Developmental Biology Division/HERL. The doses used in this study were 5, 10, 20, 40, 80, and 120 mg/kg/day. There were no live fetuses at the 120 mg/kg dose level. Dose-related decreases in gravis uterus weight and fetal weight were significant at all remaining doses of dinocap. Cleft palate was found in fetuses at 5 (0.4%), 20 (23.6%), 40 (75.7%), and 80 (74.1%) mg/kg/day. There was also a dose-related increase in supernumerary ribs, but a low frequency of exencephaly and umbilical hernia at high doses. The developmental toxicity potential of dinocap was thus demonstrated at doses well below those causing maternal toxicity (LOAEL = 80 mg/kg as characterized by significant decrease in weight gain) (HED Doc. 007456, Nov 25, 1987). The LOAEL for developmental toxicity is < 5 mg/kg/day (LDT).

Since the new formulation is the technical product with 92.2% a.i., the HIARC selected the study conducted with the new formulation.

This Risk Assessment is Required.

For General Population

Dose and Endpoint for Risk Assessment: No appropriate endpoint was identified for this population group because there were no effects attributable to a single dose was identified in oral toxicology studies including developmental toxicity studies in mice and rabbits.

B. Chronic dietary [Reference Dose (RfD)]

The following RfD was established in 1994.

Study Selected: Chronic Feeding Study - Dog **Guideline #:** 83-1b

Accession No.: 247957

Executive Summary:

In a chronic toxicity study (Accession No. 247957), technical dinocap (78% a.i.) was administered to Beagle dogs (4/sex/dose) in the diet at levels of 0, 15, 60 or varying levels of 120-240 ppm (0, 0.375, 1.5 or 3-6 mg/kg/day based on a conversion factor of 1 ppm = 0.025 mg/kg/day) for 2 years. [The test compound in the high dose group was fed at 240 ppm for 1 week, removed completely from the diet and restarted at 120 ppm for 4-30 weeks, increased again to 240 ppm for 11 days, removed completely, and then restarted at 180 ppm for 33-61 weeks.]

Treatment of dogs with varying levels of 120-240 ppm of dinocap was lethal. Four dogs (2 males and 2 females) died during the first 43 weeks. One died from unexplained causes and the other 3 deaths were related to dinocap treatment. The remaining 4 dogs had significant reduction in body weights and food consumption and they were sacrificed during week 62 because of weight loss and poor condition. Other compound related effects included occasional ataxia and clonic convulsions, salivation, decreased activity, and rapid and labored respiration.

Ocular examination revealed changes in the tapetum, retina and ocular disc of all 4 high level dogs examined (4 died before examination) and 6/8 of these dogs had histologic retinal atrophy.

The only treatment related effects noted in dogs fed 60 ppm of dinocap were **ophthalmoscopic changes** in 7/8 dogs and **histologic retinal atrophy** in 3/8 dogs.

Levels of 15 and 60 ppm of dinocap did not have an effect on oxidative phosphorylation in liver mitochondria; mitochondria from dogs in the high level were not tested.

Under the conditions of this study, dinocap did not significantly affect hematology, clinical chemistry parameters, and cholinesterase activity of plasma, RBC or brain at 15 and 60 ppm. Due to poor conditions of the high dose animals the certain clinical chemistry parameters such as albumin and globulin levels were affected.

The NOAEL for systemic toxicity is 15 ppm (0.375 mg/kg/day); the LOAEL is 60 ppm (1.5 mg/kg/day) based on ophthalmoscopic changes and histologic retinal atrophy.

This chronic feeding study in dogs **is classified as Acceptable/guideline and satisfies the guideline data requirement for a chronic toxicity study (83-1b) in dogs.**

Dose and Endpoints for Risk Assessment: The Systemic Toxicity NOAEL = 0.375 mg/kg/day and LOAEL = 1.5 mg/kg/day in male dogs, based on **ophthalmoscopic changes and histologic retinal atrophy.**

Uncertainty Factor(s): An uncertainty factor of 100 is proposed to account for inter-species extrapolation (10X) and intra-species variability (10X).

Chronic RfD: $\frac{0.375 \text{ mg/kg/day (NOAEL)}}{} = 0.00375$ g/kg/day

100 (UF)

Comments about Study/Endpoint/Uncertainty Factor(s): The endpoint was selected from the chronic dog study because dogs appear to be more sensitive species than rats or mice. Although this dog chronic study was conducted with dinocap with 78% a.i., the endpoint was selected from this because no dog chronic study is available with new formulation (92% a.i.).

C. Occupational / Residential Exposure

1. Dermal Absorption

Study Selected: Dermal Penetration Study in Monkey **Guideline #: 85-2**

Accession No.: 260614

Executive Summary:

In a dermal penetration study (Accession No. 260614), ^{14}C-ring-labeled dinocap (94% a.i.) was dermally applied to female Rhesus monkeys (4/dose) at the rate of 40 (group 2a:water-wash), 40 (group 2b:ethanol-wash) and 2500 (group 3:ethanol-wash) ug/cm^2 for 6 hours. The application area was 40, 40 and 0.64 cm^2 for groups 2a, 2b and 3, respectively. Six hours after dermal exposure, the application site was washed with water saturated cotton balls (group 2a) or ethanol laden cotton balls followed by water laden cotton balls (groups 2b and 3).

The recovery of radioactivity in 4 days following 6 hours exposure to 40 (water-wash), 40 (ethanol-wash), and 2500 (group 3:ethanol-wash) ug/cm^2 was 10.3, 5.1, and 2.6% of the applied dose in urine and 3.9, 7.3, and 1.6% of the dose in feces, respectively.

The recovery of radioactivity in dermal wash samples after 6 hours exposure to 40 (water-wash), 40 (ethanol-wash), and 2500 (group 3:ethanol-wash) ug/cm^2 was 17.5, 40.7, and 75.2% of the applied dose, respectively. The recovery data indicated that the use of ethanol as washing solution (groups 2b and 3) apparently was more effective in removing the test chemical from the application site than water.

Total recovery of radioactivity in dermal wash samples and excreta (urine and feces) in 4 days following 6 hours exposure to 40 (water-wash), 40 (ethanol-wash), and 2500 (group 3:ethanol-wash) ug/cm^2 was 49.28, 71.72, and 84.94% of the applied dose, respectively. [Note: Some radiolabel may still be retained at the original application site. However, amounts of dose remained on the skin were not measured.]

An additional excretion study of dinocap in four monkeys was conducted after a single intravenous administration of ^{14}C-ring-labeled dinocap at 0.2 mg/kg. The recovery of radioactivity in the urine and feces were 49.2 and 35.3% of the injected dose in 4 days, respectively.

Dermal absorption of dinocap in 4 days following 6 hours exposure to 40 (water-wash group), 40 (ethanol-wash group) and 2500 ug/cm^2 (ethanol-wash group) was **42.4, 20.9, and 10.6**% of the applied dose, respectively, using the following equation:

$$\text{Dermal absorption} = \frac{^{14}\text{C urinary excretion (dermal)}}{^{14}\text{C urinary excretion (i.v.)}} \times 100$$

This study is classified as acceptable/guideline and satisfies the guideline data requirement for a dermal penetration study (85-2) in monkeys.

Percentage (%) Dermal Absorption: 42.4% in 4 days following 6 hours exposure. Washing skin with water is normally done. Ethanol washing may not be appropriate in the field situation. Therefore, water wash group is preferred over ethanol wash groups.

Comments about Dermal Absorption:

From the above monkey dermal absorption study, HIARC selected 4 day excretion data instead of one day excretion data because the experimental approach utilized to determine dermal absorption preclude estimation of dermal absorption after one day. Estimation of dermal absorption using 4 day excretion data reflect proper correction for urinary excretion of dinocap following i.v. dosing.

There is a dermal penetration study (Accession No. 259639) in rabbits. This study showed that dermal absorption of dinocap (expressed as % of applied dose) in 4 days following 6 hours exposure to ^{14}C-dinocap at 25, 100, and 220 mg/kg varied from 3.8% (applied neat at 25 mg/kg) to 9.2% (wettable dust formulation at 25 mg/kg). This study would be useful when considering the possible exposure to the wettable dust formulation.

Because the permeability characteristics of monkey skin are closer to human than the rabbit, the monkey dermal absorption data is preferred over rabbit data.

2. Short-Term Dermal (1 - 7 days)

Study Selected: Developmental Toxicity Study in Mice **Guideline #:** 83-3(a)

MRID No.: 41313001

Executive Summary: See Acute Dietary.

Dose and Endpoint for Risk Assessment: The NOAEL for developmental toxicity is 4 mg/kg/day; the LOAEL is 10 mg/kg/day based on the slight (non-significant) increase in incidences of cleft palate and eyelids-open relative to the controls.

Comments about Study/Endpoint: Although a dermal developmental toxicity study in mice is available, the HIARC selected the oral study in mice because of the concern for

the developmental effects seen at lower doses, via the oral route in two studies (LOAEL = 10 mg/kg/day; < 5 mg/kg/day) compared to the dermal study (LOAEL = 100 mg/kg/day).

In addition, the endpoint was selected from the mouse developmental toxicity study because mice appear to be more sensitive species than rats or rabbits.

Also, the endpoint selection from mouse developmental toxicity is supported by a dermal developmental study in rabbits (ACCESSION Nos. 256934 and 259645). In this study, dinocap (87.8% a.i.) was dermally applied at concentrations of 0, 25, 50, or 100 mg/kg/day to presumed pregnant New Zealand white rabbits (18/dose) on gestation days (GDs) 7 through 19. Does were sacrificed on GD 29. The NOAEL for maternal toxicity is 50 mg/kg/day; the LOAEL is 100 mg/kg/day based on decreased body weights and food consumption. The NOAEL for developmental toxicity is 50 mg/kg/day; the LOAEL is 100 mg/kg/day based on increased litter and fetal incidences of skull bone islands and accessory skull bones and decreased fetal weight.

This Risk Assessment for Short-Term is Required.

3. <u>Intermediate-Term Dermal (1-Week to Several Months)</u>

Mouse developmental toxicity study Guideline #: 83-3 (a)

MRID No. 41313001

Executive Summary: Short term.

Dose and Endpoint for Risk Assessment: The NOAEL for developmental toxicity is 4 mg/kg/day; the LOAEL is 10 mg/kg/day based on the slight (non-significant) increase in incidences of cleft palate and eyelids-open relative to the controls.

Comments about Study/Endpoint: See short term.

This Risk Assessment is Required.

4. <u>Long-Term Dermal (Several Months to Lifetime)</u>

The current use pattern does not indicate a concern for Long-Term exposure or risk.
This risk assessment is **NOT** required.

5. <u>Occupational/residential Exposure–Inhalation (Any-Time Period)</u>

Except for an acute inhalation toxicity study, the results of which place DINOCAP in Toxicity Category III (LC_{50} = 0.9 mg/L), **no other studies are available via this route**. Therefore, the HIARC selected the oral NOAELs of 4 mg/kg/day from developmental toxicity study in mice for

Short-Term and Intermediate-Term inhalation risk assessments. Since an oral value is selected, route-to-route extrapolation should be as follows:

Step I. The inhalation exposure component (i.e., µg a.i/day) using a 100% absorption rate (default value) and an application rate should be converted to an **equivalent oral dose** (mg/kg/day)

Step II. The dermal exposure component (i.e., mg/kg/day) using a 42% dermal absorption value and an application rate should be converted to an **equivalent oral dose**. This dose should then be combined with the converted oral dose in Step I.

Step III. To calculate MOE's, the combined dose from Step II should then be compared to the oral NOAEL of 4 mg/kg/day for both Short-Term and Intermediate-Term exposures.

This risk assessment is required for short and intermediate term inhalation exposure.

Based on the use pattern, Long-Term inhalation exposure risk assessment is not required.

D. Recommendation for Aggregate Exposure risk Assessments

For acute aggregate exposure risk assessment, combine the high end exposure values from food + water and compare it to the acute RfD.

For short- and intermediate-term aggregate exposure risk assessment, combine the average values from food + water together with short or intermediate dermal (corrected for %DA) + short or intermediate inhalation (corrected for %IA) exposure and compared to the oral NOAEL.

Based on the use pattern, chronic aggregate exposure risk assessment is not required.

E. Margins of Exposures for Occupational/Residential Exposure Risk Assessments

A MOE of 100 is adequate for occupational exposure and the MOEs for residential (dermal and inhalation) exposure will be determined during risk characterization by the FQPA Safety Factor Committee.

III. CLASSIFICATION OF CARCINOGENIC POTENTIAL

The HED RfD/ Peer Review Committee (HED Doc. No. 011076 dated June 24, 1994) considered that the carcinogenicity phase of the rat study (MRID No. 41065401 and ACCESSION No. 00247959) and the carcinogenicity study in mice (MRID Nos. 418639801, 42079102) were considered to be adequate. The high dose levels tested in both rats and mice

were considered to be adequate for carcinogenicity testing. The treatment did not alter the spontaneous tumor profile in either animal species. The chemical was classified as a **"Group E"**.

IV. MUTAGENICITY

Dinocap was negative for inducing mutations in all acceptable guideline studies of the standard battery of mutagenicity tests except for Ames studies. In Ames studies, dinocap was weakly positive at best and limited to high doses. These studies satisfy mutagenicity testing requirements.

V. FQPA CONSIDERATIONS

1. Neurotoxicity:

There are no neurotoxicity studies available for dinocap. However, neurotoxicity in the form of clinical signs [such as swimming behavior abnormalities characterized by torticollis condition consistent with malformation of the inner ear (absence of otoliths)] was observed in treated offspring at 3 weeks of age in the 12 and 25 mg/kg/day groups in the developmental toxicity study in mice (Grey, L. E. et al. 1988: Tox. Applied Pharmacol. Vol. 92, pp.266-273). In addition, ophthalmoscopic changes and histologic retinal atrophy was observed in the chronic study in dogs (see chronic study in dogs described above; Accession No. 247957). Because of the neuropathological concerns of dinocap as shown in mice and dogs, **the HIARC recommended an acute and subchronic neurotoxicity studies be conducted. In addition, in order to further define the neurotoxic potential in the developing fetus the HIARC recommended a developmental neurotoxicity study be performed**.

2. Developmental Toxicity:

A. **Mouse Developmental Toxicity Study**

Executive Summary:

In a developmental toxicity study (MRID No. 41313001), Dinocap (94.4% a.i.) suspension in an aqueous 1% Tragacanth Gum was administered by gavage to CD-1 (ICR) BR mice (24/dose) at 0, 4, 10 or 25 mg/kg/day from gestation days 6 through 15. One group of 12 presumed pregnant females underwent caesarean section while the other 12 pregnant females were allowed to deliver naturally.

There were no maternal deaths. No clinical signs or post mortem observations were attributed to test article administration. Although not statistically significant, the body weight in the high-dose animals (25 mg/kg/day) was 5% lower than the controls on day 18 of gestation. In this group, the body weight gains were 7 and 11% lower than the

controls from gestation days 6-15 and 0-18, respectively. **The NOAEL for maternal toxicity is 10 mg/kg/day. The LOAEL for maternal toxicity is 25 mg/kg/day based on the slight decrease in body weight and body weight gains.**

Although not statistically significant, dinocap at 25 mg/kg/day caused the following: reduction in numbers of corpora lutea, implantation size, litter size and the percent of dams with any resoprtions. The number of total resorption and dead or resorbed conceptuses/litter in the high-dose animals were increased ($p<0.05$) when compared to the controls. In addition, dinocap at 25 mg/kg/day caused the following when compared to the controls: reduction in live fetal body weights (29% less than the controls, $p<0.01$); increased incidence of cleft palate ($p<0.01$), "eye lids open" ($p<0.01$), and head tilt; and an effect on swimming performance ($p<0.01$) [as measured by mice which sank and required rescue or swam on their side).

In the 10 mg/kg/day group, the number of total resorption was increased ($p<0.05$) and live fetal body weights were decreased (7% lower than the controls, $p<0.05$). However, these differences from control groups are considered to be of little or no toxicological significance. In the 10 mg/kg/day group, in addition, a slight (non-significant) increased incidences in cleft palate and eyelids-open occurred relative to the control group. Due to the absence of any occurrence in either concurrent or historical controls, the NOAEL is set conservatively at 4 mg/kg/day.

Natural delivery data showed that viability index, number of live precull on day 4 divided by number of live on day 1, was decreased ($p<0.01$) at 25 mg/kg/day (87% versus 97-100%) in the control and two lower dose groups. Lactation index was reduced ($p<0.01$) in the 4 mg/kg/day group only. However, this was primarily due to 8 pups from one litter dying by day 7 of weaning.

The NOAEL for developmental toxicity is 4 mg/kg/day; the LOAEL for developmental toxicity is 10 mg/kg/day based on the slight (non-significant) increase in incidences of cleft palate and eyelids-open relative to the controls.

This study is classified as ACCEPTABLE/NONGUIDELINE and does not satisfy the guideline data requirement for a developmental study (83-3a) in mice. The study was designed to answer specific question (the inner ear formation). The Agency requested that the registrant perform a "modified" developmental toxicity study in mice in order to elucidate developmental toxicity potential of purified dinocap (Q. Bui of the Agency to D. Edwards, dated 10/19/88, Tox. Doc. No. 007457).

B. **Rabbit Developmental Toxicity Study**

In one developmental toxicity study (ACCESSION Nos. 251713, 255892, Report No. 83R-022), dinocap (84% a.i.) in aqueous 1% (w/v) tragacanth gum was administered to pregnant New Zealand white rabbits (18/dose) by gavage at doses of 0, 3, 12, 48, or 64 mg/kg/day on gestation days (GDs) 7 through 19. In a second developmental toxicity

study (ACCESSION Nos. 252443, 255892; Report No. 83R-113), dinocap (87% a.i.) was administered by gavage to pregnant rabbits (24-48/dose) at concentrations of 0, 0.1, 0.5, or 48 mg/kg/day. These two studies were considered jointly to satisfy regulatory requirements. All does were sacrificed on GD 29.

Part 1 (Study Report No. 83R-022)

Maternal toxicity - Two controls died as a result of a dosing error. All other animals survived to scheduled sacrifice. Decreased ($p<0.1$) body weights were noted during GDs 25-29 at 12 mg/kg and body weight gains were also reduced (↓45%, not statistically significant [NS]) during GDs 7-20. Decreased fecal output was noted at dose levels of ≥12 mg/kg.

Five abortions occurred, two at 48 mg/kg and three at 64 mg/kg. When compared to concurrent controls, observations in the 48 and 64 mg/kg animals were as follows: decreased ($p<0.05$) body weights from GDs 7-29; absolute weight loss during GDs 7-20; reduced body weight gains (48 mg/kg, ↓289%; 64 mg/kg, ↓267%) during GDs 7-20; and reduced (noted as significant; no p-value provided) body weight gains on GDs 11, 15, 20, 25, and 29.

No treatment-related changes were noted in maternal necropsy observations.

Developmental toxicity - Litter size was reduced ($p<0.05$ at 12 mg/kg) in all treated groups when compared to concurrent controls. The mean number of resorptions/litter for the 0, 3, 12, 48, and 64 mg/kg groups was 0.44, 0.93, 1.33, 1.29, and 1.57, respectively; the historical control data reported a mean of 1.02. The number of litters with 2 or more resorptions was increased in the 48 and 64 mg/kg groups. Decreased ($p<0.05$) fetal weights were observed at 48 and 64 mg/kg.

During visceral examination, hydrocephaly was observed only in the treated animals; in addition, statistical analysis revealed a significant dose-response relationship, but no dose-related increase in severity was noted.

Upon skeletal examination, neural tube or skull malformations were observed at all dose levels as follows (fetuses/litter): 3 mg/kg (9/5), 12 mg/kg (4/3), 48 mg/kg (17/9), and 64 mg/kg (10/5). When malformations of the neural tube, spine, and skull were analyzed together, a dose-dependent increase was found with statistical significance noted at 3, 48, and 64 mg/kg levels; malformations included in this analysis were hydrocephaly, scoliosis, 28 presacral vertebrae, short tail, fused centra, spina bifida, small pinnae, and fused skull bones. A dose-dependent increase in the incidence of bent hyoid arch(es) was noted as follows (fetuses/litter): 3 mg/kg (2/2), 12 mg/kg (3/2), 48 mg/kg (7/5), and 64 mg/kg (10/6, statistically significant) vs 1 control fetus. The total number of fetuses with reduced ossification (hyoid, sternebrae 5 and/or 6, talus, pubis, and skull bones) was increased (statistically significant) at 48 mg/kg (27/108 fetuses and 9/14 litters) and 64 mg/kg (19/88 fetuses and 7/14 litters). Scoliosis was observed in a single 48 mg/kg fetus and 5 fetuses of 3 litters at 64 mg/kg vs 0 controls.

Part 2 (Study Report No. 83R-113)

Maternal toxicity - At study initiation, assignment to test groups was as follows: 40, 48, 48, and 24 females to the 0, 0.1, 0.5, and 48 mg/kg/day dose levels, respectively. Five deaths occurred during the study, 2 in the control group, 1 in the 0.1 mg/kg group, and 2 in the 48 mg/kg group.

Maternal body weight gains were reduced ($p<0.05$) at 48 mg/kg from GDs 7-20.

Twelve abortions occurred, two at 0.1 mg/kg, one at 0.5 mg/kg, and nine at 48 mg/kg. In addition, only 50% of the 48 mg/kg females (12/24) were pregnant at GD 29. At 48 mg/kg, an increased mean number of corpora lutea (↑17%, NS) was observed. Implantation efficiency (# implantation sites/# corpora lutea) and viability index (# live fetuses/# implantation sites) were decreased ($p<0.05$) at 48 mg/kg.

Developmental toxicity - The mean number of resorptions/litter was 0.54, 0.67, 0.54, and 1.33 for the 0, 0.1, 0.5, and 48 mg/kg (high-dose, ↑146% vs controls) levels, respectively. The number of viable fetuses/litter was reduced (↓13%, NS) at 48 mg/kg. There were no treatment-related external, visceral, or skeletal malformations or variations at any dose level. At 48 mg/kg, the number of litters and fetuses (12 litters and 80 fetuses) was small and may have concealed a teratogenic effect at this dose level.

The NOAEL for maternal toxicity is 3 mg/kg/day; the LOAEL is 12 mg/kg/day based on decreased body weights.

The NOAEL for developmental toxicity is 0.5 mg/kg/day; the LOAEL is 3 mg/kg/day based on increased incidences of hydrocephaly and malformations of neural tube, spine and skull.

When evaluated separately, each of these two studies were classified as supplementary data; however, when considered jointly, these studies are classified **acceptable/guideline** and do satisfy the guideline requirement for a developmental toxicity study in the rabbit **(§83-3[b])**.

C. Rabbit Dermal Developmental Toxicity Study

Executive Summary:

In a dermal developmental study (ACCESSION Nos. 256934 and 259645), neat dinocap (87.8% a.i.) was dermally applied at concentrations of 0, 25, 50, or 100 mg/kg/day to presumed pregnant New Zealand white rabbits (18/dose) on gestation days (GDs) 7 through 19. Does were sacrificed on GD 29. All dermal applications were made to a clipped area on the back of the rabbits and the animals were collared to prevent preening of the treated skin. Data from the range finding study indicated that the test substance could not be applied repeatedly to the same skin site without resulting in severe dermal

irritation; therefore, the test material was applied to 7 sections, each 5 x 8 cm, for a total of 40 cm^2. The test substance was removed after a 6-hour exposure by wiping the treatment site with ethanol.

Maternal toxicity - At all dose levels, dermal irritation was observed and the severity of the injury was proportional to the dose level. From GDs 8-20, dose-dependent increases (statistically significant) in erythema and edema scores were observed in the treated groups (low-dose, 0.3-2.0; mid-dose, 0.4-2.1; high-dose, 0.5-3.1; maximum score= 4) and the severity of the injury was proportional to the concentration applied. Signs of recovery were noted in all dose groups on GD 29. One 25 mg/kg doe aborted and died on GD 26 and one 50 mg/kg doe aborted on GD 23 and was subsequently sacrificed. At 100 mg/kg, clinical signs, such as, soiled anal area (6/18 treated vs 3/18 controls) and irregular feces (18/18 treated vs 10/18 controls) were noted; additionally, systemic toxicity, characterized by decreases ($p<0.05$) in food consumption (↓21%, GDs 6-20, g/day) and body weight gains (↓153%, GDs 7-20; ↓122%, GDs 0-29; ↓290%, GDs 0-20 minus fetal weight) was observed. Dermal administration of the test substance did not affect maternal survival, reproductive status, fetal weight, or percent males.

Developmental toxicity - Upon skeletal examination, treatment-related effects were observed regarding skull bone islands at 100 mg/kg. Bone islands in the skull were observed in one fetus each of the control (fetal incidence, 0.8%; litter incidence, 5.8%) and low-dose group (fetal, 0.9%; litter, 6.3%) and in 3 fetuses of 3 litters (fetal, 2.6%; litter, 21.4%) of the high-dose group; this finding was not reported in the historical control data. Above the historical control mean, accessory skull bones was observed in all treated groups at fetal and litter incidences, respectively, as follows: low-dose, 1.8 (12.5); mid-dose, 5.2 (30.0); and high-dose 0.9 (7.1) vs concurrent controls at 0 (0) and historical controls at 0.66 (4.3). The litter incidence of accessory skull bones and skull bone islands combined was 9/40 (22.5%) as compared to concurrent controls at 1/17 (5.9%) and historical controls at 6/138 (4.3%); these findings are suggestive of fetal toxicity at all dose levels tested.

Neural tube or skull malformations were reported in all treated groups with litter incidences of 11.8, 18.7, 20.0, and 21.4% in the control, 25, 50, and 100 mg/kg groups, respectively. Although these increased incidences were not statistically significant, the slight increase noted in the treated groups may suggest that a definitive teratogenic response may be observed at a higher dose level. Scoliosis with or without rib anomalies was noted only in the treated groups at a low incidence (single litter at each dose level) and was beyond the historical control mean (3/40=7.5% vs 0% of concurrent controls and 3/155= 1.9% historical controls); this finding was also observed in a dose-dependent manner in a previous oral teratology study, and therefore, the toxicological significance of scoliosis relative to dinocap exposure is unknown.

This study has undergone peer review (Accession No. 004905).

Based on local effects, the NOAEL for maternal toxicity is not established. The LOAEL for maternal toxicity is 25 mg/kg/day based on dermal irritation at the

application site at the lowest dose tested.

Based on systemic effects, the NOAEL for maternal toxicity is 50 mg/kg/day; the LOAEL is 100 mg/kg/day based on decreased body weights and food consumption.

The NOAEL for developmental toxicity is 50 mg/kg/day; the LOAEL for is 100 mg/kg/day based on increased litter and fetal incidences of skull bone islands and accessory skull bones and decreased fetal weight.

This study is classified **acceptable/guideline** and does satisfy the guideline requirement for a dermal developmental study in rabbits **(§83-3[b])**.

3. Reproductive Toxicity:

Executive Summary

In a 2-generation reproduction study (MRID No. 41542501), Dinocap (96% a.i.) was administered to Crl:CD BR(VAF/Plus) rats (26/sex/dose) in the diet at levels of 0, 40, 200 or 1000/400 ppm (0, 2.6-3.7, 12.9-18.0 or 65.1-77.3/32.4-38.7 mg/kg/day). Because of increased pup mortality during the first 7 days post-lactation, the 1000 ppm was reduced to 400 ppm in the F1 pups at weaning (week 19 of the study).

The body weights of **F0 high-dose males** were 4-8% lower than controls ($p<0.01$) throughout the study. In this group, the body weight gains were 12, 12 and 10% ($p<0.01$) lower than the controls at the 0-6, 0-10, and 0-19 week periods, respectively and food consumption were statistically significantly lower than controls at the 0-1, 1-2, 2-3, 3-4 and 6-7 week periods. During the premating period, the body weights of **F0 high-dose females** were 6-7% lower than controls ($p<0.05$ or 0.01) from weeks 6 through 10. In this group, the body weight gains were 26 and 20% lower ($p<0.01$) than controls at the 0-6 and 0-10 week periods, respectively and food consumption were statistically significantly lower than controls at 0-1 and 3-10 week periods.

During gestation in **F0 high-dose females**, the body weights were 6-7% lower than controls ($p<0.05$ or 0.01) at days 0, 6, 10 and 15, and food consumption was statistically significantly lower than controls at 0-6, 6-10 and 10-15 day periods. During lactation in **F0 high-dose females**, the body weights were 6-9% lower than controls ($p<0.05$ or 0.01) at 0, 4, 7 and 14 days.

The body weights of **F1 high-dose males** were 44, 41, 15, 9, 9, 7 and 7% lower than controls ($p<0.05$ or 0.01) at 0, 1, 6, 10, 16, 22 and 28 week periods, respectively. Food consumption was statistically significantly lower than controls at weeks 1-8 and 14-16. The body weights of **F1 high-dose females** were 37-41% lower than controls ($p<0.01$) at weeks 0 and 1.

During gestation in **F1 high-dose females**, the body weights were 8-9% lower ($p<0.05$ or 0.01) than respective control values at all measuring intervals, and food consumption

was statistically significantly lower than controls at days 0-6. During lactation in **F1 high-dose females,** the body weights were 5-9% lower than respective control values ($p<0.05$ or 0.01) at all measuring intervals.

The test chemical did not significantly affect any of the reproductive parameters.

The NOAEL for reproductive toxicity is equal to or greater than 1000/400 ppm (65.1-77.3/32.4-38.7 mg/kg/day, HDT) and the LOAEL is not established.

In **F1 offspring** in 1000 ppm group, pup weights were 24, 28 and 32% less than control values at days 7, 14 and 21, respectively. In **F2 offspring** in 400 ppm group (highest concentration), liveborn pups delivered, live pups on day 0 (birth) of lactation, and live pups on day 4 (precull) were 15, 17 and 7% lower than controls, respectively.

In high-dose group, relative liver weights were 6-8% higher ($p<0.05$) than respective control values in F0 males, F0 females and F1 females. In addition, in F0 high-dose males**,** relative testes weights were 8% higher ($p<0.05$) than respective control values.

The NOAEL for parental toxicity is 200 ppm (12.9-18.0 mg/kg/day) and the LOAEL is 1000/400 ppm (65.1-77.3 / 32.4-38.7 mg/kg/day) based on decreased body weight gain.

The NOAEL for offspring toxicity is 200 ppm (12.9-18.0 mg/kg/day) and the LOAEL is 1000/400 ppm (65.1-77.3 / 32.4-38.7 mg/kg/day) based on increased pup mortality during the first 7 days post-lactation (F1 offspring, 1000 ppm), decreased pup weight/litter, and reduced number of pups/litter.

This study is classified as Acceptable/Guideline and satisfies the guideline data requirement for a multi-generation reproduction study (83-4) in rats.

4. Additional information from the literature

There are no additional neurotoxicity studies or developmental neurotoxicity studies via inhalation or any other routes from the published literature.

5. Determination of Susceptibility

The data provided indication of increased susceptibility in mice and rabbits to *in utero* exposure to dinocap. In the prenatal developmental toxicity study in mice, while the LOAEL for maternal toxicity was 25 mg/kg/day, the LOAEL for developmental toxicity was 10 mg/kg/day. The NOAELs were 10 mg/kg/day and 4 mg/kg/day, respectively. Developmental toxicity was based on the slight (non-significant) increase in incidences of cleft palate and eyelids-open relative to the controls.

In the prenatal developmental toxicity study in rabbits, while the LOAEL for maternal toxicity

was 12 mg/kg/day, the LOAEL for developmental toxicity was 3 mg/kg/day. The NOAELs were 3 mg/kg/day and 0.5 mg/kg/day, respectively. Developmental toxicity was based on increased incidences of hydrocephaly and malformations of neural tube, spine, and skull.

In the two-generation reproduction study in rats, effects in the offspring were observed only at or above treatment levels which resulted in evidence of parental toxicity.

6. Recommendation for a Developmental Neurotoxicity Study

Based on the available data, the HIARC concluded that a developmental neurotoxicity study **is recommended.**

i. Evidence that suggest requiring a developmental neurotoxicity study include treatment-related anomalies in the development of the fetal nervous system were observed in the prenatal developmental toxicity study in mice at 10 mg/kg/day was seen in the presence of maternal toxicity at 25 mg/kg/day as indicated by slight (non-significant) increase in incidences of cleft palate and eyelids-open relative to the controls. In addition, in the prenatal developmental toxicity study in rabbits, evidence of developmental toxicity at 3 mg/kg/day was seen in the presence of maternal toxicity at 12 mg/kg/day as indicated by increased incidences of hydrocephaly and malformations of neural tube, spine and skull**.**

In addition, neurotoxicity in the form of clinical signs [such as swimming behavior abnormalities characterized by torticollis condition consistent with malformation of the inner ear (absence of otoliths)] was observed in treated offspring at 3 weeks of age in the 12 and 25 mg/kg/day groups in the developmental toxicity study in mice (Grey, L. E. et al. 1988: Tox. Applied Pharmacol. Vol. 92, pp.266-273). Also, ophthalmoscopic changes and histologic retinal atrophy was observed in the chronic study in dogs (see chronic study in dogs described above; Accession No. 247957).

In order to further define the neurotoxic potential in the developing fetus the HIARC recommended a developmental neurotoxicity study be conducted.

7. Determination of the FQPA Safety Factor:

The HIARC, based on hazard assessment alone, recommends that the additional 10 x factor be retained because of the following:

Developmental toxicity study showed increased sensitivity in fetuses as compared to maternal animals following *in utero* exposures in mice and rabbits and the need for a developmental neurotoxicity study in rabbits.

The final recommendation on the FQPA Safety Factor will be made by the FQPA Safety Committee.

VI. HAZARD CHARACTERIZATION

The toxicity data indicate that dinocap has low acute oral, dermal and inhalation toxicity. It is a skin sensitizer. It causes moderate eye and skin irritation. The chronic feeding toxicity study in rats demonstrated that dinocap induced liver toxicity (increased relative liver weights and slight to moderate panlobular hepatocellular hypertrophy) and thyroid toxicity (increased incidences of thyroid follicular cell hypertrophy and altered colloid). The chronic feeding toxicity study in dogs demonstrated that dinocap induced ophthalmoscopic changes and histologic retinal atrophy. The carcinogenicity data showed that carcinogenic potential was not exhibited by dinocap in rats and mice.

Dinocap produced developmental toxicity in mice and rabbits and it did not affect reproductive parameters in rats. Dinocap was negative for inducing mutations in all acceptable guideline studies of the standard battery of mutagenicity tests except for Ames studies. In Ames studies, dinocap was weakly positive at best and limited to high doses.

Dermal absorption studies showed that dermal absorption of dinocap (expressed as % of applied dose) in 4 days following 6 hours exposure to ^{14}C-dinocap was 42.4% and 9.2% in monkeys and rabbits, respectively.

There is high confidence in the chronic RfD of 0.00375 mg/kg/day. This was based on the NOAEL of 0.375 mg/kg/day from the chronic study in dog and uncertainty factor of 100. The LOAEL for 1.5 mg/kg/day in male dogs was based on **ophthalmoscopic changes and histologic retinal atrophy.**

The database is adequate to evaluate FQPA assessment and consists of developmental studies in the mice and rabbits, and a two generation reproduction study in the rats. Based on the findings in the developmental toxicity study in mice and rabbits, there appears to be an increased severity of effects noted in the offspring at maternally toxic doses. In addition, neurotoxicity in the form of clinical signs [such as swimming behavior abnormalities characterized by torticollis condition consistent with malformation of the inner ear (absence of otoliths)] was observed in the developmental toxicity study in rats. Also, ophthalmoscopic changes and histologic retinal atrophy was observed in the chronic study in dogs. In addition, a 30-month chronic/carcinogenicity study in rat (1980 study) showed an increased incidence of degeneration/atrophy of the calf muscle in the mid and high dose (10 and 100 mg/kg/day) females and degeneration/atrophy of the sciatic nerve in the high dose (100 mg/kg/day) males and females (MRID No. 41065401; Accession No. 247959).

VII. DATA GAPS

Acute and subchronic neurotoxicity studies in rats and a developmental neurotoxicity study in rabbit have been recommended by the HIARC.

VIII. ACUTE TOXICITY

Acute Toxicity of Dinocap

Guideline No.	Study Type	MRID No.	RESULTS	TOXICITY CATEGORY
870.1100	Acute Oral - Rat	42124301	LD 50 = > 500 and 5,000 mg/kg (both sexes)	III
870.1200	Acute Dermal - Rabbit	42124302	LD50 => 5,000 mg/kg	IV
870.1300	Acute Inhalation - Rat	42124303	LC50= 0.9 mg/L	III
870.2400	Eye Irritation- Rabbit	42124304	moderate irritation; Corneal and conjunctival effects at 24 hours; corneal effects cleared by day 2 and conjunctival effects cleared by day 7.	III
870.2500	Dermal Irritation- Rabbit	42124305	moderate irritation at day 3 and cleared by day 14.	III
870.2600	Dermal sensitization - Guinea pig	42124306	a sensitizer	N/A

IX. SUMMARY OF TOXICOLOGY ENDPOINT SELECTION

The doses and toxicological endpoints selected for various exposure scenarios are summarized below.

EXPOSURE SCENARIO	DOSE (mg/kg/day)	ENDPOINT	STUDY
Acute Dietary (Female 13+)	NOAEL=4	slight (non-significant) increase in incidences of cleft palate and eyelids-open	Developmental–mouse MRID No. 41313001
	UF=100	**Acute RfD = 0.04 mg/kg/day**	
Acute Dietary (General population)	No endpoint established		
Chronic Dietary	NOAEL=0.375	ophthalmoscopic changes and histologic retinal atrophy	Chronic feeding –dog ACCESSION No. 247957
	UF=100	**Chronic =0.00375 mg/kg/day**	
Short-Term [(a)] (Dermal)	oral NOAEL=4	slight (non-significant) increase in incidences of cleft palate and eyelids-open	Developmental–mouse MRID No. 41313001
Intermediate-Term (Dermal)	oral NOAEL=4	**slight (non-significant) increase in incidences of cleft palate and eyelids-open**	**Developmental–mouse MRID No. 41313001**
Long-Term (Dermal)	**Not required under the registered use patterns**		
Inhalation (short & intermediate) [(b)]	**oral NOAEL=4**	**slight (non-significant) increase in incidences of cleft palate and eyelids-open**	**Developmental–mouse MRID No. 41313001**
Inhalation (long)	**None**	**Not required under the registered use patterns**	

a = Since an oral NOAEL was selected, a dermal absorption factor of 42% should be used in route-to-route extrapolation.

b = Since an oral NOAEL was selected, an inhalation absorption factor of 100% (default value) should be used in route-to-route extrapolation.